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1	12164363	sensor or apparatus or device or equipment or machine or appliance or instrument or tool	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:21
2	2955	(sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:52
4	352506	(sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near3 (colorimetric or fluorophore or fluorescence or fluorescent or (chemo adj illumines\$) or (chemo adj lumines\$) or illumines\$ or lumines\$ or magnet\$ or radioactive or diffraction or potentiom\$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:37
5	374	((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and ((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near3 (colorimetric or fluorophore or fluorescence or fluorescent or (chemo adj illumines\$) or (chemo adj lumines\$) or illumines\$ or lumines\$ or magnet\$ or radioactive or diffraction or potentiom\$))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:37
6	19831	((detect\$ or identif\$) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:39
7	164	((detect\$ or identif\$) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and (((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and ((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near3 (colorimetric or fluorophore or fluorescence or fluorescent or (chemo adj illumines\$) or (chemo adj lumines\$) or illumines\$ or lumines\$ or magnet\$ or radioactive or diffraction or potentiom\$)))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:40

8	86	(((detect\$ or identif\$) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and (((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and ((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near3 (colorimetric or fluorophore or fluorescence or fluorescent or (chemo adj illumines\$) or (chemo adj lumines\$) or illumines\$ or lumines\$ or magnet\$ or radioactive or diffraction or potentiom\$) ))) and (@py<2002)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 13:25
9	21	(((detect\$ or identif\$) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and (((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near2 (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)) and ((sensor or apparatus or device or equipment or machine or appliance or instrument or tool) near3 (colorimetric or fluorophore or fluorescence or fluorescent or (chemo adj illumines\$) or (chemo adj lumines\$) or illumines\$ or lumines\$ or magnet\$ or radioactive or diffraction or potentiom\$) ))) and (@py<2002)) NOT ACTIVITY	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/06/22 12:55

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(DETECT\* IS NOT A RECOGNIZED COMMAND  
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=> s (detect\* or identify\*) (3A) (enzyme or protease or proteinase or peptidase or metalloprotease or metalloproteinase or metallopeptidase)  
13 FILES SEARCHED...  
24 FILES SEARCHED...  
34 FILES SEARCHED...  
51 FILES SEARCHED...  
66 FILES SEARCHED...  
87 FILES SEARCHED...

L3 21531 (DETECT\* OR IDENTIFY\*) (3A) (ENZYME OR PROTEASE OR PROTEINASE  
OR PEPTIDASE OR METALLOPROTEASE OR METALLOPROTEINASE OR METALLOP  
EPTIDASE)

=> s (detect\* or identify\* or sense or measure\* or quantify\* or determine\*) near3  
(colorimetric or fluorophore or fluorescence or fluorescent or (chemo illumines\*)  
or (chemo lumines\*) or illumines\* or lumines\* or magnet\* or radioactive or  
diffraction or potentiom\*)  
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nested terms that are not separated by a logical operator.

=> s (detect\* or identify\* or sense or measure\* or quantify\* or determine\*) (3A)  
(colorimetric or fluorophore or fluorescence or fluorescent or (chemo illumines\*)  
or (chemo lumines\*) or illumines\* or lumines\* or magnet\* or radioactive or  
diffraction or potentiom\*)  
11 FILES SEARCHED...  
21 FILES SEARCHED...  
29 FILES SEARCHED...  
42 FILES SEARCHED...  
54 FILES SEARCHED...  
63 FILES SEARCHED...  
67 FILES SEARCHED...  
81 FILES SEARCHED...  
97 FILES SEARCHED...

L4 63608 (DETECT\* OR IDENTIFY\* OR SENSE OR MEASURE\* OR QUANTIFY\* OR DETER  
MINE\*) (3A) (COLORIMETRIC OR FLUOROPHORE OR FLUORESCENCE OR  
FLUORESCENT OR (CHEMO ILLUMINES\*) OR (CHEMO LUMINES\*) OR ILLUMIN  
ES\* OR LUMINES\* OR MAGNET\* OR RADIOACTIVE OR DIFFRACTION OR  
POTENTIOM\*)

=> s (capture antibody) (4A) (protease or proteinase or peptidase or  
metalloprotease or metalloproteinase or metallopeptidase)  
20 FILES SEARCHED...  
32 FILES SEARCHED...  
55 FILES SEARCHED...  
64 FILES SEARCHED...  
93 FILES SEARCHED...

L5 30 (CAPTURE ANTIBODY) (4A) (PROTEASE OR PROTEINASE OR PEPTIDASE OR  
METALLOPROTEASE OR METALLOPROTEINASE OR METALLOPEPTIDASE)

=> s l3 and l4 and l5  
55 FILES SEARCHED...

L6 1 L3 AND L4 AND L5

=> d l6 1 bib ab

L6 ANSWER 1 OF 1 USPATFULL on STN

AN 2003:173229 USPATFULL  
 TI Sensors and methods of detection for proteinase enzymes  
 IN Quirk, Stephen, Alpharetta, GA, UNITED STATES  
 Tyrrell, David John, Appleton, WI, UNITED STATES  
 PI US 2003119073 A1 20030626  
 AI US 2001-26393 A1 20011221 (10)  
 DT Utility  
 FS APPLICATION  
 LREP JOHN S. PRATT, KILPATRICK STOCKTON LLP (KIMBERLY CLARK), 1100 PEACHTREE  
 STREET, SUITE 2800, ATLANTA, GA, 30309  
 CLMN Number of Claims: 45  
 ECL Exemplary Claim: 1  
 DRWN 7 Drawing Page(s)  
 LN.CNT 925  
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
 AB Sensors for detecting catabolic proteinase enzymes and proenzymes in the  
 fluid of a human or animal and methods for detecting the enzymes and  
 then providing treatment that is specific for the detected enzyme are  
 disclosed. The sensors of the present invention can be used to  
**detect catabolic proteinase enzymes** and  
 proenzymes in the fluid of chronic wounds of humans and animals. Upon  
 detection of any proteinase enzyme, the wound can be treated with an  
 inhibiting complex that is specific for the detected enzyme or  
 proenzyme. Enzymes such as matrix metalloproteinases and human  
 neutrophil elastase in the active and proenzyme form can be detected and  
 treatment provided with inhibitors for the detected enzyme.  
  
 => s (capture antibody)  
 24 FILES SEARCHED...  
 60 FILES SEARCHED...  
 99 FILES SEARCHED...  
 L7 7027 (CAPTURE ANTIBODY)  
  
 => s l3 and l4 and l7  
 49 FILES SEARCHED...  
 L8 13 L3 AND L4 AND L7  
  
 => duplicate  
 ENTER REMOVE, IDENTIFY, ONLY, OR (?):  
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 'L700' IS NOT VALID HERE  
 Enter "REMOVE" to identify and remove duplicate answers.  
 Enter "IDENTIFY" to identify duplicate answers in the answer set.  
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 duplicate records.  
 ENTER REMOVE, IDENTIFY, ONLY, OR (?):remove  
 ENTER L# LIST OR (END):l8  
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 DUPLICATE PREFERENCE IS 'USPATFULL, WPINDEX'  
 KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n  
 PROCESSING COMPLETED FOR L8  
 L9 13 DUPLICATE REMOVE L8 (0 DUPLICATES REMOVED)  
  
 => d l9 1-13 bib ab

L9 ANSWER 1 OF 13 USPATFULL on STN  
AN 2004:77737 USPATFULL  
TI Multiplexed analysis of clinical specimens apparatus and methods  
IN Chandler, Van S., Austin, TX, UNITED STATES  
Fulton, Jerrold R., Cedar Hill, TX, UNITED STATES  
Chandler, Mark B., Austin, TX, UNITED STATES  
PI US 2004059519 A1 20040325  
AI US 2001-971647 A1 20011009 (9)  
RLI Division of Ser. No. US 1998-286, filed on 18 Aug 1998, GRANTED, Pat.  
No. US 6449562 A 371 of International Ser. No. WO 1996-US16198, filed on  
10 Oct 1996, PENDING  
DT Utility  
FS APPLICATION  
LREP PATENT ADMINSTRATOR, KATTEN MUCHIN ZAVIS ROSENMAN, 525 WEST MONROE  
STREET, SUITE 1600, CHICAGO, IL, 60661-3693  
CLMN Number of Claims: 85  
ECL Exemplary Claim: 1  
DRWN 65 Drawing Page(s)  
LN.CNT 4841

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for the multiplexed diagnostic and genetic analysis of enzymes,  
DNA fragments, antibodies, and other biomolecules comprises the steps of  
constructing an appropriately labeled beadset, exposing the beadset to a  
clinical sample, and analyzing the combined sample/beadset by flow  
cytometry is disclosed. Flow cytometric measurements are used to  
classify, in real-time, beads within an exposed beadset and textual  
explanations, based on the accumulated data obtained during real-time  
analysis, are generated for the user. The inventive technology enables  
the simultaneous, and automated, detection and interpretation of  
multiple biomolecules or DNA sequences in real-time while also reducing  
the cost of performing diagnostic and genetic assays.

L9 ANSWER 2 OF 13 USPATFULL on STN  
AN 2004:50876 USPATFULL  
TI Compositions and methods for detection and isolation of phosphorylated  
molecules  
IN Agnew, Brian, Eugene, OR, UNITED STATES  
Beechem, Joseph, Eugene, OR, UNITED STATES  
Gee, Kyle, Springfield, OR, UNITED STATES  
Haugland, Richard, Eugene, OR, UNITED STATES  
Liu, Jixiang, Eugene, OR, UNITED STATES  
Martin, Vladimir, Eugene, OR, UNITED STATES  
Patton, Wayne, Eugene, OR, UNITED STATES  
Steinberg, Thomas, Eugene, OR, UNITED STATES  
PI US 2004038306 A1 20040226  
AI US 2003-428192 A1 20030502 (10)  
PRAI US 2002-377733P 20020503 (60)  
US 2002-393059P 20020628 (60)  
US 2002-407255P 20020830 (60)  
US 2003-440252P 20030114 (60)  
DT Utility  
FS APPLICATION  
LREP KOREN ANDERSON, MOLECULAR PROBES, INC., 29851 WILLOW CREEK ROAD, EUGENE,  
OR, 97402-9132  
CLMN Number of Claims: 74  
ECL Exemplary Claim: 1  
DRWN 12 Drawing Page(s)  
LN.CNT 5760

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to phosphate-binding compounds that find  
use in binding, detecting and isolating phosphorylated target molecules  
including the subsequent identification of target molecules that  
interact with phosphorylated target molecules or molecules capable of

being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target molecule and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target molecule resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target molecules, isolation of phosphorylated target molecules from a complex mixture and aiding in proteomic analysis wherein kinase and phosphatase substrates and enzymes can be identified.

L9 ANSWER 3 OF 13 USPATFULL on STN  
AN 2003:237907 USPATFULL  
TI Compositions and methods for the therapy and diagnosis of colon cancer  
IN King, Gordon E., Shoreline, WA, UNITED STATES  
Meagher, Madeleine Joy, Seattle, WA, UNITED STATES  
Xu, Jiangchun, Bellevue, WA, UNITED STATES  
Secrist, Heather, Seattle, WA, UNITED STATES  
Jiang, Yuqiu, Kent, WA, UNITED STATES  
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)  
PI US 2003166064 A1 20030904  
AI US 2002-99926 A1 20020314 (10)  
RLI Continuation-in-part of Ser. No. US 2001-33528, filed on 26 Dec 2001,  
PENDING Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul  
2001, PENDING  
PRAI US 2001-302051P 20010629 (60)  
US 2001-279763P 20010328 (60)  
US 2000-223283P 20000803 (60)  
DT Utility  
FS APPLICATION  
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,  
SEATTLE, WA, 98104-7092  
CLMN Number of Claims: 17  
ECL Exemplary Claim: 1  
DRWN No Drawings  
LN.CNT 8531  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB Compositions and methods for the therapy and diagnosis of cancer,  
particularly colon cancer, are disclosed. Illustrative compositions  
comprise one or more colon tumor polypeptides, immunogenic portions  
thereof, polynucleotides that encode such polypeptides, antigen  
presenting cell that expresses such polypeptides, and T cells that are  
specific for cells expressing such polypeptides. The disclosed  
compositions are useful, for example, in the diagnosis, prevention  
and/or treatment of diseases, particularly colon cancer.

L9 ANSWER 4 OF 13 USPATFULL on STN  
AN 2003:173229 USPATFULL  
TI Sensors and methods of detection for proteinase enzymes  
IN Quirk, Stephen, Alpharetta, GA, UNITED STATES  
Tyrrell, David John, Appleton, WI, UNITED STATES  
PI US 2003119073 A1 20030626  
AI US 2001-26393 A1 20011221 (10)  
DT Utility  
FS APPLICATION  
LREP JOHN S. PRATT, KILPATRICK STOCKTON LLP (KIMBERLY CLARK), 1100 PEACHTREE  
STREET, SUITE 2800, ATLANTA, GA, 30309  
CLMN Number of Claims: 45  
ECL Exemplary Claim: 1  
DRWN 7 Drawing Page(s)  
LN.CNT 925  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Sensors for detecting catabolic proteinase enzymes and proenzymes in the fluid of a human or animal and methods for detecting the enzymes and then providing treatment that is specific for the detected enzyme are disclosed. The sensors of the present invention can be used to **detect catabolic proteinase enzymes** and proenzymes in the fluid of chronic wounds of humans and animals. Upon detection of any proteinase enzyme, the wound can be treated with an inhibiting complex that is specific for the detected enzyme or proenzyme. Enzymes such as matrix metalloproteinases and human neutrophil elastase in the active and proenzyme form can be detected and treatment provided with inhibitors for the detected enzyme.

L9 ANSWER 5 OF 13 USPATFULL on STN

AN 2003:106233 USPATFULL

TI Compositions and methods for the therapy and diagnosis of pancreatic cancer

IN Benson, Darin R., Seattle, WA, UNITED STATES  
Kalos, Michael D., Seattle, WA, UNITED STATES  
Lodes, Michael J., Seattle, WA, UNITED STATES  
Persing, David H., Redmond, WA, UNITED STATES  
Hepler, William T., Seattle, WA, UNITED STATES  
Jiang, Yuqiu, Kent, WA, UNITED STATES

PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

PI US 2003073144 A1 20030417

AI US 2002-60036 A1 20020130 (10)

PRAI US 2001-333626P 20011127 (60)  
US 2001-305484P 20010712 (60)  
US 2001-265305P 20010130 (60)  
US 2001-267568P 20010209 (60)  
US 2001-313999P 20010820 (60)  
US 2001-291631P 20010516 (60)  
US 2001-287112P 20010428 (60)  
US 2001-278651P 20010321 (60)  
US 2001-265682P 20010131 (60)

DT Utility

FS APPLICATION

LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,  
SEATTLE, WA, 98104-7092

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 14253

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.

L9 ANSWER 6 OF 13 USPATFULL on STN

AN 2003:71556 USPATFULL

TI High throughput or capillary-based screening for a bioactivity or biomolecule

IN Short, Jay M., Rancho Santa Fe, CA, UNITED STATES  
Keller, Martin, San Diego, CA, UNITED STATES  
Lafferty, William Michael, Encinitas, CA, UNITED STATES

PI US 2003049841 A1 20030313

AI US 2001-975036 A1 20011010 (9)

RLI Continuation-in-part of Ser. No. US 2001-894956, filed on 27 Jun 2001,  
PENDING Continuation-in-part of Ser. No. US 2001-790321, filed on 21 Feb



2001, PENDING Continuation-in-part of Ser. No. US 2000-687219, filed on 12 Oct 2000, PENDING Continuation-in-part of Ser. No. US 2000-685432, filed on 10 Oct 2000, PENDING Continuation-in-part of Ser. No. US 1999-444112, filed on 22 Nov 1999, PENDING Continuation-in-part of Ser. No. US 1998-98206, filed on 16 Jun 1998, GRANTED, Pat. No. US 6174673 Continuation-in-part of Ser. No. US 1997-876276, filed on 16 Jun 1997, PENDING

PRAI US 2001-309101P 20010731 (60)  
DT Utility  
FS APPLICATION  
LREP Lisa A. Haile, Ph.D., Gray Cary Ware & Freidenrich LLP, Suite 1100, 4365 Executive Drive, San Diego, CA, 92121-2189  
CLMN Number of Claims: 211  
ECL Exemplary Claim: 1  
DRWN 27 Drawing Page(s)  
LN.CNT 6452

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Provided is a method of screening or enriching a sample containing polynucleotides from a mixed population of organisms. The method includes creating a DNA library from a plurality of nucleic acid sequences of a mixed population of organisms and separating clones containing a polynucleotide sequence of interest on an analyzer detects a detectable molecule on a probe or bioactive substrate. The analyzer includes FACS devices, SQUID devices and MCS devices. The separated or enrich library can then be further process by activity based screening or sequence based screening. In addition, the enriched sequence can be compared to a database and to identify sequences in the database which have homology to a clone in the library thereby obtaining a nucleic acid profile of the mixed population of organisms.

L9 ANSWER 7 OF 13 USPATFULL on STN  
AN 2002:243051 USPATFULL  
TI Compositions and methods for the therapy and diagnosis of ovarian cancer  
IN Algate, Paul A., Issaquah, WA, UNITED STATES  
Jones, Robert, Seattle, WA, UNITED STATES  
Harlocker, Susan L., Seattle, WA, UNITED STATES  
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)  
PI US 2002132237 A1 20020919  
AI US 2001-867701 A1 20010529 (9)  
PRAI US 2000-207484P 20000526 (60)  
DT Utility  
FS APPLICATION  
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092  
CLMN Number of Claims: 11  
ECL Exemplary Claim: 1  
DRWN No Drawings  
LN.CNT 25718

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

L9 ANSWER 8 OF 13 USPATFULL on STN  
AN 2002:242791 USPATFULL  
TI Compositions and methods for the therapy and diagnosis of colon cancer  
IN King, Gordon E., Shoreline, WA, UNITED STATES  
Meagher, Madeleine Joy, Seattle, WA, UNITED STATES

Xu, Jiangchun, Bellevue, WA, UNITED STATES  
Secrist, Heather, Seattle, WA, UNITED STATES  
PA Corixa Corporation, Seattle, WA, UNITED STATES (U.S. corporation)  
PI US 2002131971 A1 20020919  
AI US 2001-33528 A1 20011226 (10)  
RLI Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul 2001,  
PENDING  
PRAI US 2001-302051P 20010629 (60)  
US 2001-279763P 20010328 (60)  
US 2000-223283P 20000803 (60)  
DT Utility  
FS APPLICATION  
LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,  
SEATTLE, WA, 98104-7092  
CLMN Number of Claims: 17  
ECL Exemplary Claim: 1  
DRWN No Drawings  
LN.CNT 8083

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

L9 ANSWER 9 OF 13 USPATFULL on STN  
AN 2002:231977 USPATFULL  
TI Multiplexed analysis of clinical specimens apparatus and method  
IN Chandler, Van S., Austin, TX, United States  
Fulton, Jerrold R., Cedar Hill, TX, United States  
Chandler, Mark B., Austin, TX, United States  
PA Luminex Corporation, Austin, TX, United States (U.S. corporation)  
PI US 6449562 B1 20020910  
WO 9714028 19970417  
AI US 1998-286 19980818 (9)  
WO 1996-US16198 19961010  
19980818 PCT 371 date

DT Utility  
FS GRANTED  
EXNAM Primary Examiner: Fredman, Jeffrey  
LREP Villacorta, Gilberto M., Pepper Hamilton LLP  
CLMN Number of Claims: 13  
ECL Exemplary Claim: 3  
DRWN 77 Drawing Figure(s); 65 Drawing Page(s)  
LN.CNT 4019

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for the multiplexed diagnostic and genetic analysis of enzymes, DNA fragments, antibodies, and other biomolecules comprises the steps of constructing an appropriately labeled beadset, exposing the beadset to a clinical sample, and analyzing the combined sample/beadset by flow cytometry is disclosed. Flow cytometric measurements are used to classify, in real-time, beads within an exposed beadset and textual explanations, based on the accumulated data obtained during real-time analysis, are generated for the user. The inventive technology enables the simultaneous, and automated, detection and interpretation of multiple biomolecules or DNA sequences in real-time while also reducing the cost of performing diagnostic and genetic assays.

L9 ANSWER 10 OF 13 WPINDEX COPYRIGHT 2004 THOMSON DERWENT on STN  
AN 2002-106198 [14] WPINDEX

DNN N2002-079008                      DNC C2002-032595  
 TI Isolated antigenic human or mouse DNAX receptor subunit-like polypeptide  
 useful for detecting antibodies generated in response to presence of  
 increased protein levels or immunological disorders.  
 DC B04 D16 S03  
 IN GORMAN, D M  
 PA (SCHE) SCHERING CORP; (GORM-I) GORMAN D M  
 CYC 95  
 PI WO 2001090358    A2 20011129 (200214)\* EN 148  
     RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ  
         NL OA PT SD SE SL SZ TR TZ UG ZW  
     W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CZ DE DK DM  
         DZ EC EE ES FI GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT  
         LU LV MA MD MG MK MN MX MZ NO NZ PL PT RO RU SE SG SI SK SL TJ TM  
         TR TT TZ UA UZ VN YU ZA  
 AU 2001074920    A 20011203 (200221)  
 EP 1303604        A2 20030423 (200329) EN  
     R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT  
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 US 2003092881    A1 20030515 (200335)  
 CN 1444652        A 20030924 (200382)  
 JP 2003534013    W 20031118 (200401)                      183  
 MX 2002011617    A1 20030301 (200413)  
 ADT WO 2001090358 A2 WO 2001-US16767 20010523; AU 2001074920 A AU 2001-74920  
 20010523; EP 1303604 A2 EP 2001-941579 20010523, WO 2001-US16767 20010523;  
 US 2003092881 A1 Provisional US 2000-206862P 20000524, US 2001-863818  
 20010523; CN 1444652 A CN 2001-813327 20010523; JP 2003534013 W JP  
 2001-587152 20010523, WO 2001-US16767 20010523; MX 2002011617 A1 WO  
 2001-US16767 20010523, MX 2002-11617 20021122  
 FDT AU 2001074920 A Based on WO 2001090358; EP 1303604 A2 Based on WO  
 2001090358; JP 2003534013 W Based on WO 2001090358; MX 2002011617 A1 Based  
 on WO 2001090358  
 PRAI US 2000-206862P                      20000524; US 2001-863818                      20010523  
 AB WO 200190358 A UPAB: 20020301  
 NOVELTY - A substantially pure or isolated antigenic polypeptide (I) which  
 comprises a fully defined:  
     (a) human DNAX cytokine receptor subunit-like 8 (DCRS8) polypeptide  
 of 722 amino acids (S1); or  
     (b) human DCRS9 polypeptide sequence of 634 amino acids (S2); or  
     (c) mouse DCRS9 polypeptide sequence of 220 amino acids (S3), is new.  
 All sequences are as given in specification.  
 DETAILED DESCRIPTION - A antigenic polypeptide (I) comprising:  
     (a) a substantially pure or recombinant polypeptide comprising at  
 least three distinct non overlapping segments of at least four amino acids  
 identical to segments of (S1)-(S3);  
     (b) a substantially pure or recombinant polypeptide comprising at  
 least two distinct nonoverlapping segments of at least five amino acids  
 identical to segments of (S1)-(S3);  
     (c) a natural sequence DCRS8 comprising mature (S1)-(S3); and/or  
     (d) a fusion polypeptide comprising DCRS8 or DCRS9 sequence.  
 INDEPENDENT CLAIMS are also included for the following:  
     (1) a composition comprising:  
         (a) a substantially pure DCRS8 and DCRS9 and another cytokine  
 receptor family member;  
         (b) a sterile (I);  
         (c) (I) and a carrier;  
     (2) a kit comprising (I) and a compartment comprising the protein or  
 polypeptide, or instructions for use or disposal of reagents in the kit;  
     (3) a binding compound (II) comprising an antigen binding site from  
 an antibody which specifically binds to (I), where:  
     (a) the binding compound is in a container;  
     (b) the DCRS8 or DCRS9 polypeptide is from a human;  
     (c) the binding compound is an Fv, Fab, or Fab2 fragment;

- (d) the binding compound is conjugated to another chemical group; or
- (e) the antibody:
  - (i) is raised against peptide sequence of a mature polypeptide of (S1)-(S4), mature DCRS8 or DCRS9, purified human DCRS8 or DCRS9;
  - (ii) is immunoselected;
  - (iii) is a polyclonal antibody;
  - (iv) binds to a denatured DCRS8 or DCRS9;
  - (v) exhibits Kd to antigen of at least 30 mu M;
  - (vi) is attached to a solid substrate, including a bead or plastic membrane;
  - (vii) is in a sterile composition; or
  - (viii) is detectably labeled, including a radioactive or fluorescent label;
- (4) a kit comprising (II) and a compartment comprising (II), or instructions for use or disposal of reagents in the kit;
- (5) producing an antigen:antibody complex, involves contacting under appropriate conditions a primate DCRS8 or DCRS9 polypeptide (II), thereby allowing the complex to form;
- (6) a composition comprising (II), or (II) and a carrier;
- (7) an isolated or recombinant nucleic acid (III) encoding (I), where the DCRS8 or DCRS9 is from a human, or the nucleic acid:
  - (a) encodes one or several antigenic peptide sequence of (S1)-(S4);
  - (b) exhibits identity over at least thirteen nucleotides to a natural cDNA encoding the segment;
  - (c) is an expression vector;
  - (d) further comprises an origin of replication;
  - (e) is from a natural source;
  - (f) comprises a detectable label;
  - (g) comprises synthetic nucleotide sequence;
  - (h) is less than 6 kb, preferably less than 3 kb;
  - (i) is from a primate;
  - (j) comprises a natural full length coding sequence;
  - (k) is a hybridization probe for a gene encoding DCRS8 or DCRS9; or
  - (l) is a PCR primer, PCR product, or mutagenesis primer;
  - (8) a cell or tissue (IV) comprising (III);
  - (9) a kit comprising (III), and a compartment comprising the nucleic acid, a compartment further comprising a primate DCRS8 or DCRS9 polypeptide, or instructions for use or disposal of reagents in the kit;
  - (10) a nucleic acid (V) which hybridizes under stringent conditions to a coding portion of a fully defined human DCRS8 polynucleotide sequence of 2786 nucleotides or human DCRS9 polynucleotide sequence of 2012 nucleotides as given in the specification, or exhibits identity over a stretch of at least about 30 nucleotides to a primate DCRS8 or DCRS9; and
  - (11) modulating (M1) physiology or development of a cell or tissue culture cells involves contacting the cell with an agonist or antagonist of a mammalian DCRS8 or DCRS9.

ACTIVITY - None given.

MECHANISM OF ACTION - Gene therapy; protein therapy; vaccine. Agonist or antagonist of mammalian DCRS8 or DCRS9.

USE - The receptors, or its portions may be useful as phosphate labeling enzymes to label general or specific substrates. The subunits may also be functional immunogens to elicit recognizing antibodies, or antigens capable of binding antibodies. A combination, e.g., including a DCRS8 can be used as an immunogen for the production of antisera or antibodies specific, e.g., capable of distinguishing between other cytokine receptor family members, for the combinations described. The purified DCRS8 can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of expression, or immunological disorders which lead to antibody production to the endogenous receptor.

Additionally, DCRS8 fragments may also serve as immunogens to produce the antibodies. A nucleic acid which codes for the DCRS8 or DCRS9 is useful to identify genes, mRNA, and cDNA species which code for itself or

closely related proteins, as well as DNAs which code for polymorphic, allelic, or other genetic variants, e.g., from different individuals or related species. Antibodies against DCRS8 or DCRS9 can be used in competitive drug screening assays, e.g., where neutralizing antibodies to the receptor complexes or fragments compete with a test compound for binding to a ligand or other antibody.

Therefore the neutralizing antibodies or fragments can be used to detect the presence of a polypeptide which shares one or more binding sites to a receptor and can also be used to occupy binding sites on a receptor that might otherwise bind a ligand. The antibodies, including antigen binding fragments, are potent antagonists that bind to the receptor and inhibit binding to ligand or inhibit the ability of the receptor to elicit a biological response, e.g., act on its substrate.

The antibodies can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they bind to the receptor without inhibiting ligand or substrate binding. The antibodies can also be used for affinity chromatography in isolating the DCRS8 proteins or peptides and to screen expression libraries for particular expression products.

Dwg.0/0

L9 ANSWER 11 OF 13 USPTFULL on STN  
AN 2000:34437 USPTFULL  
TI Raman spectroscopic method for determining the ligand binding capacity of biologicals  
IN Grow, Ann E., 5882 Highplace Dr., San Diego, CA, United States 92120  
PI US 6040191 20000321  
AI US 1998-177548 19981022 (9)  
RLI Division of Ser. No. US 1997-864015, filed on 27 May 1997, now patented, Pat. No. US 5866430  
PRAI US 1996-19742P 19960613 (60)  
DT Utility  
FS Granted  
EXNAM Primary Examiner: Weber, Jon P.  
LREP Beehler & Pavitt  
CLMN Number of Claims: 11  
ECL Exemplary Claim: 1  
DRWN 20 Drawing Figure(s); 8 Drawing Page(s)  
LN.CNT 3869

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A nondestructive process for determining the reactive capacity of a test biological by Raman scattering. The test biological may be any one of enzymes, enzyme cofactors, coenzymes, antibodies, antibody fragments, hemeproteins, peptides, synthetic peptides, toxins, toxoids, glycosphingolipids, lectins, lipids, lipid complexes, phospholipids, carbohydrates, saccharides, gangliosides, nucleic acids, fragments of nucleic acids, pathogen adhesion factors, receptors, receptor subunits, membranes, organelles, cells, tissues and complexes containing membranes, organelles, cells and tissues, or a bioconcentrator. The test biological is irradiated with a light source to produce a Raman scattering spectrum of the irradiated biological. The Raman scattering spectrum is collected and processed to determine the ability of the test biological to react with ligands. The analyzing step includes comparing the Raman scattering spectrum of the test biological against that of a biological standard of the same biological which has been altered to vary the capability to react with ligands thereby determining the capacity of the test biological to react with ligands.

L9 ANSWER 12 OF 13 USPTFULL on STN  
AN 1999:15786 USPTFULL  
TI Raman optrode processes and devices for detection of chemicals and microorganisms  
IN Grow, Ann E., 5882 Highplace Dr., San Diego, CA, United States 92120  
PI US 5866430 19990202

AI US 1997-864015 19970527 (8)  
PRAI US 1996-19742P 19960613 (60)  
DT Utility  
FS Granted  
EXNAM Primary Examiner: Weber, John P.  
LREP Beehler & Pavitt  
CLMN Number of Claims: 24  
ECL Exemplary Claim: 1  
DRWN 20 Drawing Figure(s); 8 Drawing Page(s)  
LN.CNT 3934

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A methodology and devices for detecting or monitoring or identifying chemical or microbial analytes are described. The methodology comprises four basic steps: (1) The gas or liquid medium to be monitored or analyzed is brought into contact with a bioconcentrator which is used to bind with or collect and concentrate one or more analytes. (2) The bioconcentrator-analyte complex is then exposed to radiation of one or more predetermined wavelengths to produce Raman scattering spectral bands. (3) At least a portion of the Raman spectral bands are collected and processed by a Raman spectrometer to convert the same into an electrical signal. And (4) the electrical signal is processed to detect and identify, qualitatively and/or quantitatively, the analyte(s). The methodology of this invention may also comprise Raman reactive capacity analysis of the bioconcentrator itself, simultaneously with or independently from the detection of the analyte, to determine the potential ability of the bioconcentrator to complex with analytes; the results of this latter analysis may be used to affect or alter or modify the methodology involved in detection and analysis of the analytes. Also the invention is accomplished by a Raman Optrode comprising: a bioconcentrator capable of binding with the analyte(s); a mechanism or procedure or device for bringing the gas or liquid sample into contact with the bioconcentrator; a light source suitable for generating Raman scattering; a Raman spectrometer capable of collecting and processing the Raman scattering spectral information and translating it into an electrical signal; and electronic hardware and software for analyzing the electrical signal and translating the signal into information on the presence, identity and/or quantity of the bound analytes. Various forms of bioconcentrators are described, as well as a variety of analytes which may be detected, monitored, or identified by this invention, and a variety of devices which can be fabricated based on this invention.

L9 ANSWER 13 OF 13 USPATFULL on STN

AN 91:40461 USPATFULL

TI Fluorescent detection method based on enzyme activated conversion of a fluorophore precursor substrate

IN Harte, Richard A., Redwood City, CA, United States

Mastin, Stephen H., Rockville, MD, United States

PA Microbiological Associates, Inc., Rockville County, MD, United States  
(U.S. corporation)

PI US 5017475 19910521

AI US 1987-89605 19870825 (7)

RLI Continuation-in-part of Ser. No. US 1985-801671, filed on 25 Nov 1985,  
now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Rosen, Sam

LREP White, John P.

CLMN Number of Claims: 29

ECL Exemplary Claim: 1

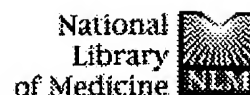
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 1013

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods are provided for the fluorescent detection of an analyte of

interest wherein an amine-substituted, ortho-fused pyrazine fluorophore, with neither nitrogen of the pyrazine ring being fused or substituted, is produced by enzymatic oxidation of a fluorophore precursor substrate which comprises a nitrogen-substituted, cyclic compound. Also provided are novel fluorophore-labelled compounds.



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A new Lp(a) assay that is unaffected by apo(a) size polymorphism.  
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 PMID: 10509894 [PubMed - indexed for MEDLINE]
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Equivalent recognition of free and ACT-complexed PSA in a monoclonal-polyclonal sandwich assay is conferred by binding specificity of the monoclonal antibody.  
 J Clin Lab Anal. 1998;12(4):242-9.




PMID: 9671177 [PubMed - indexed for MEDLINE]


☐ **8:** [Matta H, Puni V, Kanwar SS.](#) [Related Articles](#), [Link](#)

 **An immuno-dot blot assay for detection of thermostable protease from *Pseudomonas* sp. AFT-36 of dairy origin.**  
Lett Appl Microbiol. 1997 Oct;25(4):300-2.  
PMID: 9351281 [PubMed - indexed for MEDLINE]


☐ **9:** [Ostergaard PB, Beck TC, Orsted H, Svendsen A, Nordfang O, Sandset PM, Hansen JB.](#) [Related Articles](#), [Link](#)

 **An enzyme linked immunosorption assay for tissue factor pathway inhibitor**  
Thromb Res. 1997 Sep 1;87(5):447-59.  
PMID: 9306619 [PubMed - indexed for MEDLINE]


☐ **10:** [Philippou H, Adami A, Amersey RA, Stubbs PJ, Lane DA.](#) [Related Articles](#), [Link](#)

 **A novel specific immunoassay for plasma two-chain factor VIIa: investigation of FVIIa levels in normal individuals and in patients with acute coronary syndromes.**  
Blood. 1997 Feb 1;89(3):767-75.  
PMID: 9028306 [PubMed - indexed for MEDLINE]


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 **Thrombin production, inactivation and expression during open heart surgery measured by assays for activation fragments including a new ELISA for prothrombin fragment F1 + 2.**  
Thromb Haemost. 1993 Aug 2;70(2):253-8.  
PMID: 8236130 [PubMed - indexed for MEDLINE]


☐ **12:** [Boisclair MD, Lane DA.](#) [Related Articles](#), [Link](#)

 **A microtitre plate ELISA to measure thrombin-antithrombin complex using pan-specific antibodies.**  
Blood Coagul Fibrinolysis. 1992 Dec;3(6):795-802.  
PMID: 1489901 [PubMed - indexed for MEDLINE]

☐ **13:** [Kaufman N, Page JD, Pixley RA, Schein R, Schmaier AH, Colman RW.](#) [Related Articles](#), [Link](#)

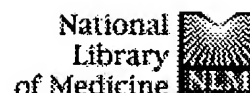
 **Alpha 2-macroglobulin-kallikrein complexes detect contact system activation in hereditary angioedema and human sepsis.**  
Blood. 1991 Jun 15;77(12):2660-7.  
PMID: 1710516 [PubMed - indexed for MEDLINE]

☐ **14:** [Declerck PJ, Lijnen HR, Verstreken M, Moreau H, Collen D.](#) [Related Articles](#), [Link](#)

 **A monoclonal antibody specific for two-chain urokinase-type plasminogen activator. Application to the study of the mechanism of clot lysis with single-chain urokinase-type plasminogen activator in plasma.**  
Blood. 1990 May 1;75(9):1794-800.  
PMID: 1691934 [PubMed - indexed for MEDLINE]

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### Analysis of collagenase-cleavage of type II collagen using a neopeptide ELISA.

Downs JT, Lane CL, Nestor NB, McLellan TJ, Kelly MA, Karam GA, Mezes PS, Pelletier JP, Otterness IG.

Inflammation Biology, Pfizer Central Research, Pfizer Inc., Groton, CT 06340-8002, USA. james\_t\_downs@groton.pfizer.com

We have developed monoclonal antibody 5109 against a unique highly acidic sequence in type II collagen. When paired with previously reported monoclonal antibody 9A4, 5109 can be used as the capture antibody in an ELISA assay for the neopeptide generated by collagenase-cleavage of type II collagen. The assay detects the sequence ZGlyGluX(759) GlyAspAspGlyProSerGlyAlaGluGlyProX(771)GlyProGlnGly(775) where Z is a variable length polypeptide, X is proline or hydroxyproline, and Gly(775) corresponds to C-terminal amino acid of the 3/4 piece after collagenase cleavage. Antibody 5109 detects the first and 9A4 the second underlined sequence. Antibody 5109 recognizes its epitope with a  $K=1.2 \times 10^{-8}$  M independently of hydroxylation of X(759). When X(771) is proline, the sequence is 90x more sensitively detected by this ELISA than when it is hydroxyproline. Type II collagen of human articular cartilage was fragmented by cyanogen bromide (CNBr) and trypsin. The immunoreactive fragment was captured with 5109 and sequenced. Proline(771) averaged 81% hydroxylated. Other 3rd position prolines were >97% hydroxylated. In urine of control individuals of 50-70 years of age, we failed to detect the presence of the collagen fragment in a majority (8/10) of specimens. The two controls with measurable levels averaged 123 pM. In a similar age cohort of osteoarthritic patients, the majority (9/10) showed measurable values of urinary collagen fragments averaging 312 pM. This assay can be used for monitoring type II collagen metabolism in patients with osteoarthritis.

PMID: 11150534 [PubMed - indexed for MEDLINE]

2: Microbiol Res. 2000 Sep;155(3):197-203.

[Related Articles](#), [Link](#)

**An immunoassay for detection of heat-stable proteases from thermophilic psychrotrophic *Bacillus* spp. of dairy origin.**

**Matta H, Punj V.**

Department of Microbiology, College of Basic Sciences, Himachal Pradesh Agricultural University, Palampur, India.

A homogeneous preparation of a thermostable protease from *Bacillus* sp. B-17 was used to raise an antiserum in rabbits. IgG of this antiserum was used to study the antigenic relationship of proteases in cell-free extracts of 21 bacilli of milk origin. Based on immunological cross reactivity, the 21 bacilli were divided into 3 serological subgroups. To raise antibodies of broader specificity, protease from *Bacillus* sp. B-11 (group II) and B-3 (group III) were purified, mixed with purified B-17 protease, and an antiserum was raised against this mixture. IgG of this antiserum was purified (IgG anti-bacilli protease). A sandwich ELISA was standardized using IgG anti-bacilli protease as capture antibody. The assay could detect 1.2 ng ml<sup>-1</sup> of protease in milk or buffer, but the assay failed to detect 4 of 21 bacilli proteases. The results suggest that this assay is useful for the detection of proteases of *Bacillus* spp. in dairy industry.

PMID: 11061187 [PubMed - indexed for MEDLINE]

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3: Thromb Haemost. 2000 Jun;83(6):874-81.

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**A monoclonal antibody raised against human beta-factor XIIa which also recognizes alpha-factor XIIa but not factor XII or complexes of factor XIIa with C1 esterase inhibitor.**

**Esnouf MP, Burgess AI, Dodds AW, Sarphie AF, Miller GJ.**

Nuffield Department of Clinical Biochemistry, University of Oxford, The Radcliffe Infirmary, UK.

A monoclonal antibody (mAb 2/215) against human beta-factor XIIa (beta-FXIIa), was shown by equilibrium binding studies to have a high affinity for alpha-factor XIIa (alpha-FXIIa) (K<sub>d</sub> 1.8 nM) and beta-FXIIa (K<sub>d</sub> 0.65 nM) but no detectable reaction with FXII zymogen or alpha-FXIIa:C1 esterase inhibitor (C1-INH) complex. Surface plasmon resonance studies showed that the mAb 2/215 bound to immobilized alpha-FXIIa with high affinity (K<sub>D</sub> 3.93 +/- 1.46 x 10<sup>-11</sup> M). Western blots employing mAb 2/215 indicated that human plasma contained small amounts of alpha-FXIIa but no beta-FXIIa. mAb 2/215 did not inhibit the amidolytic activity of beta-FXIIa and protected beta-FXIIa from inhibition by C1-INH. The recovery by ELISA, employing mAb 2/215 as the capture antibody, of alpha-FXIIa added

to plasma was 11.3%, 42% after inhibition of alpha-FXIIa with 3:4dichloroisocoumarin, and 82% when 0.5% Triton-X100 was added to the assay. Gel filtration showed that the majority of plasma alpha-FXIIa existed as a complex ( $M_r$  approximately 170,000). This distinctive mAb increases the capacity to study the contact system in health and disease.

PMID: 10896241 [PubMed - indexed for MEDLINE]

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4: Clin Chem. 2000 Jan;46(1):38-46.

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### **Measurement of different forms of tissue plasminogen activator in plasma.**

**Chandler WL, Jascur ML, Henderson PJ.**

Department of Laboratory Medicine, University of Washington, Seattle, WA 98195, USA. [wlc@u.washington.edu](mailto:wlc@u.washington.edu)

**BACKGROUND:** We evaluated assays to measure both total tissue plasminogen activator (tPA) and the three principle forms of tPA in plasma: active tPA, tPA complexed with plasminogen activator inhibitor type 1 (PAI-1), and tPA complexed with C1-inhibitor. **METHODS:** Active tPA was measured by use of an indirect amidolytic assay and immunofunctional assays. tPA/PAI-1, tPA/C1-inhibitor, and total tPA antigen were measured by use of microtiter plates coated with anti-tPA antibodies and, respectively, anti-PAI-1, anti-C1-inhibitor, and anti-tPA antibodies conjugated to peroxidase. **RESULTS:** The immunofunctional tPA assay detected 1 U/L (0.001 U/mL) tPA and recovered 108% +/- 12% of active tPA added to samples containing high (mean, 60 000 IU/L) PAI-1 activities vs a detection limit of 10 U/L (0.01 U/mL) and 13% +/- 25% recovery for the indirect amidolytic tPA activity assay. For measurement of tPA/PAI-1 complex, polyclonal anti-PAI-1 conjugates recovered 112% +/- 20% of the expected tPA/PAI-1 vs recovery of only 38% +/- 16% when monoclonal anti-PAI-1 conjugates were used. Of three methods tested, two total tPA antigen assays correlated well ( $r(2) = 0.85$ ) and showed recoveries near 100%, whereas the third method showed lower correlations, higher intercepts, and falsely high recovery. A single anti-tPA capture antibody that performed the best in the individual assay evaluations was used to measure the different forms of tPA in 22 samples with a range of tPA and PAI-1 values. The sum of the molar concentrations of active tPA, tPA/PAI-1, and tPA/C1-inhibitor using the optimized methods was equal to 94% +/- 7% of measured total tPA. **CONCLUSION:** Optimized assays based on a single anti-tPA capture antibody can be used to accurately measure the major forms of tPA in plasma.

PMID: 10620570 [PubMed - indexed for MEDLINE]

5: Clin Chim Acta. 1999 Sep;287(1-2):29-43.

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Erratum in:

- Clin Chim Acta 2000 Feb 25;292(1-2):187.

**A new Lp(a) assay that is unaffected by apo(a) size polymorphism.**

**Yamada S, Inoue K, Morishita R, Ogihara T, Kubono K, Kubo N, Abe A, Sakurabayashi I.**

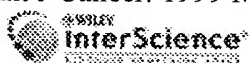
Central Institute, Shinotest Corporation, Sagamihara-shi, Kanagawa-ken, Japan.

We developed sandwich ELISA methods in which anti-apo(a) kringle 4 type 5 through protease (K4 x 5-Pro) domain monoclonal antibody (clone: 203E2) is employed in each instance as the capture antibody and one of the three species of monoclonal antibody [Mab] (clones: 108B8, 202A9, 2B3) is used as the labeled antibody. Using serum containing apo(a) with 34 repeats of kringle 4 as the calibrator, a commercial kit using anti-Lp(a) polyclonal antibody (Pab) or anti-apo(a) Mab overestimated the Lp(a) concentration in samples containing apo(a) with more than 34 repeats of kringle 4 and underestimated the Lp(a) concentration in samples containing apo(a) with fewer than 34 repeats of kringle 4. Moreover, it was demonstrated that the ratios of commercial kit values to anti-apo(a) K4 x 5-Pro Mab-based method values increased as the size of apo(a) increased. The ratios of apo(a) K5 x Pro Mab-based method values to anti-apo(a) K4 x 5-Pro Mab-based method values, however, remained almost constant regardless of the size polymorphism. Thus, we suggest that apo(a) size heterogeneity can significantly affect Lp(a) measurement in the Lp(a) assay using anti-Lp(a) Pab. The novel Lp(a) assay method, using only anti-apo(a) K4 x 5-Pro Mab, is not subject to this phenomenon.

PMID: 10509894 [PubMed - indexed for MEDLINE]

6: Int J Cancer. 1999 May 17;81(4):598-606.

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**ELISA for complexes of urokinase-type and tissue-type plasminogen activators with their type-1 inhibitor (uPA-PAI-1 and tPA-PAI-1).**

**Grebenschikov N, Sweep F, Geurts A, Andreasen P, De Witte H, Schousboe S, Heuvel J, Benraad T.**

Department of Chemical Endocrinology, University Hospital Nijmegen St. Radboud, The Netherlands.

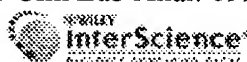
An ELISA has been developed for the assessment of complexes between the urokinase-type (uPA) and the tissue-type plasminogen (tPA) activators with their inhibitor type-1 (PAI-1) in cell-culture medium and cytosolic extracts of breast tumours. The "4-stage/2-site" ELISA involves 2 polyclonal antibodies in the pre-analyte stage 2 and in the post-analyte stage. For the specific measurement of the uPA-PAI-1 complex, 2 assay formats may be employed, uPA/PAI-1 and PAI-1/uPA. This offers an attractive facility for quality-assessment studies of this kind of assays. Analogously, the tPA-PAI-1 complex was assessed using the formats tPA/PAI-1 and PAI-1/tPA. Only complexes are able to evoke a signal in their appropriate assay formats. The free component, however, which responds to the capture antibody, could interfere with the binding of the complex molecule, reducing the OD signal. Increasing the coating Ab concentration diminishes the signal-suppressing effect of the free component. In 15 cell-culture supernatants, uPA and PAI-1 concentrations were measured as well as the uPA of PAI-1 complex in different dilutions in 2 assay formats. The differences between the values of complex measured in the 2 assay formats could be accounted for by the free uPA and PAI-1 concentrations. At dilution 1:10, the measured values obtained in the 2 separate formats differed substantially (correlation coefficient  $r = 0.641$ ). At dilution 1:20, the differences were already smaller between the values (agreement 0.945). At dilution 1:30, close agreement between the corresponding values was observed ( $r = 0.971$ ). Extrapolation to infinite dilution of the results obtained resulted in an even closer estimation of the complex concentration. Comparable results have been observed when tPA, PAI-1 and tPA-PAI-1 values were measured in tumour biopsy extracts.

PMID: 10225451 [PubMed - indexed for MEDLINE]

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☐ 7: J Clin Lab Anal. 1998;12(4):242-9.

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**Equivalent recognition of free and ACT-complexed PSA in a monoclonal-polyclonal sandwich assay is conferred by binding specificity of the monoclonal antibody.**

**Zhou Z, Armstrong EG, Belenky A, Freeman JV, Yeung KK.**

Business Group Diagnostics, Immunodiagnostics Business Unit, Bayer Corporation, Tarrytown, New York 10591-5097, USA.

The Bayer Immuno 1 PSA Assay measures free and ACT-complexed PSA on an equimolar basis, although it uses a monoclonal antibody (MM1) for capture and polyclonal antibodies for detection. Competitive inhibition studies using antibodies directed at various epitopes on PSA and PSA-ACT demonstrated that the capture antibody, MM1, does not bind to free PSA simultaneously with antibodies against Epitope E which is exposed only in free PSA. Affinity studies showed that the affinity constants of MM1 for both free PSA and PSA-ACT are similar. One explanation for the properties of

MM1 is that it precludes the binding of antibodies to Epitope E due to steric hindrance. Alternatively, the binding of MM1 causes a conformation change within the free PSA molecule, so that Epitope E is altered in a way that cause a loss of binding affinity. The unusual properties of MM1 are responsible for the equimolar response of this monoclonal-polyclonal sandwich assay for free and ACT-complexed PSA.

PMID: 9671177 [PubMed - indexed for MEDLINE]

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8: Lett Appl Microbiol. 1997 Oct;25(4):300-2.

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**An immuno-dot blot assay for detection of thermostable protease from *Pseudomonas* sp. AFT-36 of dairy origin.**

**Matta H, Punj V, Kanwar SS.**

Department of Microbiology, College of Basic Sciences, Himachal Pradesh Agricultural University, Palampur, India.

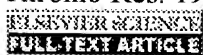
A dot-ELISA technique for the detection of *Pseudomonas* protease was developed using IgG of anti-*Pseudomonas* AFT-36 protease as capture antibody. The detection limit of protease in buffer or milk was 1.01 ng ml<sup>-1</sup>. The procedure was performed at room temperature, took about 2.5 h and was economical. Protease AFT-36 is immunologically related to five out of seven *Pseudomonas* spp. The results suggest that the assay could be used to detect proteases in dairy products.

PMID: 9351281 [PubMed - indexed for MEDLINE]

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9: Thromb Res. 1997 Sep 1;87(5):447-59.

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**An enzyme linked immunosorption assay for tissue factor pathway inhibitor.**

**Ostergaard PB, Beck TC, Orsted H, Svendsen A, Nordfang O, Sandset PM, Hansen JB.**

Novo Nordisk A/S, Bagsvaerd, Denmark.

An assay for the quantification of full-length and carboxy-terminus truncated tissue factor pathway inhibitor (TFPI) has been developed. The assay is a classical two-antibody sandwich assay with a monoclonal capture antibody directed against the third Kunitz-type domain of human TFPI and a polyclonal rabbit peroxidase-labelled anti-human TFPI detecting antibody. The assay is sensitive to full-length and carboxy-terminus truncated TFPI

with intact third Kunitz-type domain, but not to two-domain TFPI. TFPI associated with lipoproteins is not or only sparsely detected and TFPI in complex with factor Xa only partially measured. The assays gives linear reference curves in the dose range of 5 to 100 ng/ml in a double logarithmic plot. The normal range assessed from analyses on citrated plasma from 81 normal human donors is 7.8 to 26.0 ng/ml (average  $\pm$  2 SD, log-normal distribution). There is no statistically significant difference between TFPI levels measured in 10 fasting and 10 non-fasting individuals. The reproducibility of the assay is about 5.6-5.9% (relative standard error) and the within-days and between-day reproducibilities are 4.7-5.1% and 5.9-8.5%, respectively. The assay is in very good agreement with a commercial ELISA assay recently marketed. A robust, reproducible and convenient ELISA assay for the determination of full-length and three-domain TFPI has been developed.

PMID: 9306619 [PubMed - indexed for MEDLINE]

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☐ 10: Blood. 1997 Feb 1;89(3):767-75.

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**A novel specific immunoassay for plasma two-chain factor VIIa: investigation of FVIIa levels in normal individuals and in patients with acute coronary syndromes.**

**Philippou H, Adami A, Amersey RA, Stubbs PJ, Lane DA.**

Department of Haematology, Charing Cross and Westminster Medical School, London, UK.

We report the development of an enzyme-linked immunosorbent assay (ELISA) that is specific for factor VIIa (FVIIa). This assay uses a neoantigen specific capture antibody directed to the amino acid peptide sequence N terminal to the FVII cleavage activation site. The antibody exhibits approximately 3,000-fold greater reactivity to FVIIa than FVII on a molar basis. Experiments using plasma with added (exogenous) human FVIIa gave quantitative recovery in the ELISA over a range of 0.20 to 3.2 ng/mL of FVIIa. The intra- and inter-assay coefficient of variation (CVs) of the ELISA are 4.5% and 9.8%, respectively. The ELISA shows excellent correlation ( $r = .99$ ) with a functional assay (using recombinant soluble tissue factor) in detecting FVIIa added to plasma over the range 0.05 to 18.0 ng/mL. However, a major discrepancy exists between the two assays when normal endogenous plasma concentrations of FVIIa are measured. Using normal plasma ( $n = 14$ ) the functional assay reported  $3.10 \pm 0.30$  ng/mL (mean  $\pm$  SE) whereas only  $0.025 \pm 0.010$  ng/mL was detected in the same samples by the immunoassay. Patients ( $n = 43$ ) presenting with acute coronary syndrome (myocardial infarction and unstable angina) exhibited elevations ( $P < .05$ ) in immunologically detected FVIIa,  $0.093 \pm 0.013$  ng/mL (mean  $\pm$  SE) compared to patient controls ( $n = 20$ ) contemporaneously admitted with



noncardiac chest pain,  $0.048 \pm 0.007$  ng/mL (mean  $\pm$  SE). These elevations in the acute coronary syndromes were accompanied by increased ( $P < .05$ ) and correlating prothrombin fragment F1 + 2 levels (Spearman correlation coefficient  $r_s = .4$ ,  $P < .01$ ), demonstrating that thrombin generation is certainly associated with, and may even be caused by, extrinsic pathway activation.

PMID: 9028306 [PubMed - indexed for MEDLINE]

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11: Thromb Haemost. 1993 Aug 2;70(2):253-8.

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**Thrombin production, inactivation and expression during open heart surgery measured by assays for activation fragments including a new ELISA for prothrombin fragment F1 + 2.**

**Boisclair MD, Lane DA, Philippou H, Sheikh S, Hunt B.**

Department of Haematology, Charing Cross & Westminster Medical School, London, United Kingdom.

Activation of coagulation was studied during the peri-operative period in patients undergoing cardiopulmonary bypass (CPB) surgery using activation markers which have recently become available: prothrombin fragment F1 + 2 (F1 + 2), which is a measure of total thrombin generation, and thrombin-antithrombin complex, which is a measure of inactivation of free thrombin by antithrombin. Levels of the specific marker of fibrin breakdown, D-dimer, were also determined. F1 + 2 levels were assessed using a newly developed ELISA described herein which employs a neoantigen-specific capture antibody raised using a synthetic peptide; the latter antibody has been pre-adsorbed against prothrombin to ensure high specificity for F1 + 2. Increased generation of thrombin during surgery was clearly demonstrated despite maintenance of a high concentration of heparin during the period of extracorporeal blood circulation. There was a close association ( $r = 0.882$ ) between the generation of thrombin (F1 + 2 levels) and its inhibition (TAT levels). Differences were noted, however, between the information provided by F1 + 2 and TAT, which are interpreted with regard to the different in vivo fates of F1 + 2 and thrombin. The enhanced activation and inhibition of coagulation observed during CPB was suppressed once physiological blood circulation was restored, with F1 + 2 returning to pre-surgical levels within 2 h after surgery. During the post-operative period D-dimer levels, which rose in concert with F1 + 2 and TAT levels, remained highly elevated, suggesting that not all of the generated thrombin was inactivated by antithrombin. (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 8236130 [PubMed - indexed for MEDLINE]

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12: Blood Coagul Fibrinolysis. 1992 Dec;3(6):795-802.

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**A microtitre plate ELISA to measure thrombin-antithrombin complex using pan-specific antibodies.**

**Boisclair MD, Lane DA.**

Department of Haematology, Charing Cross & Westminster Medical School, London, UK.

A sandwich enzyme-linked immunosorbent assay (ELISA) has been developed to measure plasma levels of thrombin-antithrombin complex (TAT). The assay is performed in a microtitre plate using polyclonal antibodies specific for antigenic determinants on prothrombin and antithrombin. Antibody to prothrombin was immobilized on a solid phase, using a titre predetermined to optimize capture of TAT. The performance of the microtitre plate ELISA for TAT has been extensively investigated and compared with the performance characteristics of a tube-based ELISA for TAT which is available commercially (Enzygnost-TAT, from Behringwerke, Marburg, Germany). Studies with plasma containing various levels of prothrombin showed that the zymogen competed with TAT for capture antibody in both assays. Variations in prothrombin levels between plasma samples present a potential source of artifact, but one which does not critically affect the performance of either assay in detecting large elevations in TAT. A high correlation ( $r = 0.88$ ) was established between the results of plasma samples assayed by both assays, whether citrate or EDTA anticoagulant was used to prepare plasma. High correlations ( $r > 0.90$ ) were also established for each assay between the results of plasma prepared with EDTA as compared to citrate anticoagulant. Both assays were able to discriminate completely between a group of 16 normal controls and a group of 31 patients with disseminated intravascular coagulation (DIC).

PMID: 1489901 [PubMed - indexed for MEDLINE]

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13: Blood. 1991 Jun 15;77(12):2660-7.

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**Alpha 2-macroglobulin-kallikrein complexes detect contact system activation in hereditary angioedema and human sepsis.**

**Kaufman N, Page JD, Pixley RA, Schein R, Schmaier AH, Colman RW.**

Thrombosis Research Center, Temple University School of Medicine, Philadelphia PA 19140.

Activation of the contact system has been documented in severe sepsis and hereditary angioedema, but a sensitive, specific, and quantitative assay for assessing the degree of involvement of this proteolytic enzyme cascade is not yet available. We have developed a quantitative sandwich enzyme-linked

immunosorbent assay (ELISA) for the alpha 2-macroglobulin-kallikrein (alpha 2M-Kal) complex using an F(ab')<sub>2</sub> derivative of a monospecific polyclonal antibody against alpha 2 M as the capture antibody and a unique murine monoclonal antibody, 13G11, against the heavy chain of kallikrein as the detector antibody. The assay does not detect complexes in normal plasma but reacts with complexes generated by activating normal plasma with dextran sulfate at 4 degrees C in a range of 5 to 375 nmol/L. A close correlation of the ELISA with an amidolytic assay for alpha 2M-Kal was documented. Patients with sepsis syndrome but negative bacterial blood cultures did not show elevated plasma complexes, whereas a majority of those with positive blood cultures did show modest elevation and a single patient with septic shock showed a very high level of alpha 2M-Kal complex. Similarly, a patient with classic hereditary angioedema (HAE) showed increased concentration of complexes on three separate occasions during attacks but normal levels between attacks. Two other HAE patients did not show elevated levels at quiescent periods. The ELISA for alpha 2M-Kal appears to be sensitive, specific, and quantitative, and it can be used to reflect the degree of contact system activation in human sepsis and in HAE attacks.

PMID: 1710516 [PubMed - indexed for MEDLINE]

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❑ 14: Blood. 1990 May 1;75(9):1794-800.

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**A monoclonal antibody specific for two-chain urokinase-type plasminogen activator. Application to the study of the mechanism of clot lysis with single-chain urokinase-type plasminogen activator in plasma.**

**Declerck PJ, Lijnen HR, Verstreken M, Moreau H, Collen D.**

Center for Thrombosis and Vascular Research, University of Leuven, Belgium.

A murine monoclonal antibody (MA-12E6A8) was raised against human urokinase-type plasminogen activator (u-PA), which, in an enzyme-linked immunosorbent assay (ELISA), reacted 15,000-fold better with recombinant two-chain u-PA (rtcu-PA) than with recombinant single-chain u-PA (rscu-PA). The antibody had no effect on the activity of rtcu-PA or on its inhibition by a chloromethylketone, but reduced the inhibition of rtcu-PA by recombinant plasminogen activator inhibitor-1 (rPAI-1) at least 10-fold. The dissociation constant of the rtcu-PA/MA-12E6A8 complex was 7 nmol/L. An ELISA was developed using MA-12E6A8 as capture antibody and a horseradish peroxidase conjugated u-PA specific antibody for tagging. It recognized free and active site blocked rtcu-PA but not rtcu-PA in complex with rPAI-1 or with alpha 2-antiplasmin. This ELISA was used to monitor the generation of rtcu-PA during fibrin clot lysis with rscu-PA in human plasma. Addition of 5 micrograms/mL rscu-PA to 3 mL plasma containing a 0.2 mL 125I-fibrin labeled plasma clot caused 50% clot lysis in 62 +/- 13 minutes

(mean  $\pm$  SD,  $n = 6$ ), at which time  $99 \pm 28$  ng/mL rtcu-PA was detected but no fibrinogen breakdown had occurred. Fifty percent fibrinogen breakdown did occur only when rtcu-PA had reached a level of  $1,000 \pm 27$  ng/mL (at  $150 \pm 21$  minutes). rscu-PA, 2 micrograms/mL, induced 50% clot lysis in  $160 \pm 41$  minutes ( $n = 6$ ); no fibrinogen degradation occurred within 4 hours and rtcu-PA levels did not exceed 80 ng/mL. In the absence of a fibrin clot, 5 micrograms/mL rscu-PA added to human plasma did not result in significant generation of rtcu-PA (less than 50 ng/mL after 4 hours) and no fibrinogen degradation was observed. These results indicate that clot lysis with rscu-PA in a plasma milieu does not require extensive systemic conversion of rscu-PA to rtcu-PA, and that fibrinogen degradation occurs secondarily to systemic conversion of rscu-PA to rtcu-PA.

PMID: 1691934 [PubMed - indexed for MEDLINE]

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